CONVERSION OF PTEROYLMONOGLUTAMATE TO 5-METHYLTETRAHYDROFOLATE BY THE RAT JEJUNUM

EDWARD JAY OLINGER

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CONVERSION OF PTEROYLMONOGLUTAMATE TO 5-METHYLTETRAHYDROFOLATE BY THE RAT JEJUNUM

by

Edward Jay Olinger

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Grateful acknowledgment is made to Dr. Howard Spiro, for three years my friend and mentor.

I am also indebted to Mrs. Arlene Cashmore, Mrs. Judy Uhoch, Dr. Paul Chello and Claudia Rawlins for their expert assistance and good humor.

I wish to express gratitude to the American Society of Clinical Nutrition for their financial support of this work.

Finally, this acknowledgment would be incomplete without mentioning the support and encouragement of my wife.

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SUMMARY

Studies were designed to determine whether pteroylmonoglutamate (PGA) at physiologic concentrations is transported across the small intestine unaltered or is reduced and methylated to the circulating folate form during absorption. ^{3}H-PGA at varying concentrations was incubated on the mucosal side of rat jejunum placed between lucite chambers. Of the folate transferred to the serosal side, the percent identified as 5-methyltetrahydrofolate (5-MTHF) by DEAE-Sephadex chromatography was inversely related to the initial mucosal PGA concentration: at 7, 20 and 2000 nM, 46%, 32% and $<4\%$ respectively was 5-MTHF. In contrast, at 20 nM the ileum converted $<4\%$ to 5-MTHF. 10^{-6}M methotrexate, a dihydrofolate (DHF) reductase inhibitor, markedly inhibited PGA conversion to 5-MTHF by the jejunum. DHF reductase activity was measured in homogenates of villus scrapings to determine if the difference in PGA conversion to 5-MTHF between jejunum and ileum might be explained by differences in this enzyme activity. The purity of the villus preparation was confirmed by a 5 fold increase in sucrase specific activity (S.A.) and a 20 fold decrease in thymidine kinase S.A. compared to mucosal homogenates. A significant difference in DHF reductase S.A. in jejunal (0.105 ± 0.012 μmole/hr/mg protein) compared to ileal villi (0.036 ± 0.003) was found. These studies demonstrate that both the reduction and methylation of PGA to 5-MTHF and DHF reductase S.A. is greater in the jejunum than in the ileum. The initial reduction

of PGA to tetrahydrofolate by DHF reductase may be the rate limiting step. These results could explain previous studies which have failed to identify conversion of PGA at higher and less physiologic concentrations.

INTRODUCTION

The absorption of pteroylglutamic acid (folic acid, PGA) in man after oral administration has been demonstrated by studies measuring plasma urine and fecal levels of folate derivatives[1-10]. Clinical[11-15] and experimental[16-20] evidence favors the jejunum as the primary site of folic acid absorption although one study[21] in the rat finds no difference between jejunal and ileal PGA absorption. However, the mechanism by which PGA and its derivatives are transported across the small intestine is still uncertain.

To understand the transport of folate*, it is useful to review qualitatively and quantitatively the distribution of folate derivatives in the diet and in the circulation. Analysis of food stuffs has revealed that between 80 and 90% of natural folates exist as polyglutamyl conjugates with unreduced and unconjugated PGA representing between 1 and 10% of the total dietary folate (500-700 μg)[29,30]. Moreover, the predominant circulating and storage forms of folate are reduced forms, principally 5-methyltetrahydrofolate (5-MTHF)[65].

The data concerning the intestinal absorption of conjugated folate (figure 6) suggests that the polyglutamate is quantitatively deconjugated before release into the circulation[9,31-35] although two studies[36,37] give evidence for the

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*refers to all forms of folate

absorption of the intact heptaglutamate. In addition, the finding of negligible conjugase activity in the intestinal brush border[38] has left the anatomical site of polyglutamate deconjugation in doubt. One study in rats[28] has elegantly demonstrated by a variety of techniques that for 5-MTHF, intestinal transport is by a non-energy dependent non-saturable system.

Despite our knowledge that 10% or less of dietary folate is PGA, most studies have dealt with the absorption of this compound alone[16-21,23-27,45,46] since it is stable and available commercially. These investigations have presented conflicting data concerning the mechanism of PGA transfer across the intestine. Although several studies in rats[16,21,23] and hamsters[24,25] have demonstrated active transport as the process governing PGA absorption, other studies in the rat[19,20,26] and hamster[27] have not demonstrated active transport and have suggested that PGA is absorbed by passive diffusion.

Following oral PGA administration, the plasma 5-MTHF level increases[7,39,45,46]. Therefore PGA is converted to 5-MTHF at one or more sites between the point of ingestion and the peripheral circulation. Baker, et. al.[7] suggested that folic acid was converted to 5-MTHF during transfer across the intestine, and studies in humans[39] and with everted intestinal sacs[22] in the rat[40] and hamster[25] are in agreement with this thesis. In addition, the finding of DHF reductase activity in the small intestine[41-43] and its localization to the crypts of Lieberkuhn and the villus as determined by autoradiographic techniques[44]

support those observations. However, in vivo perfusion studies of isolated intestinal segments in dogs[35], experiments with everted intestinal sacs in rats[20] and human studies[45,46] involving hepatic and umbilical vein catheterization give evidence that PGA crosses the intestine intact without metabolic conversion.

The experiments failing to demonstrate reduction and methylation of PGA during absorption must be understood in light of the fact that conversion may have been masked by the pharmacologic doses of PGA used in these studies[20,35,45,46] and that measures to protect reduced folates from light[35] and aerobic conditions[20,35,45,46] appear not to have been adequately taken.

It is the purpose of this study, utilizing recently developed chromatographic methods and the apparatus described by Schultz and Zalusky[47], to determine whether PGA at physiologic concentrations is converted to other folate forms during transfer across the rat intestinal mucosa, and if so, to study some of the characteristics of this conversion.

MATERIALS AND METHODS

MATERIALS

Glucose oxidase, peroxidase, and o-dianisidine were purchased from Sigma, St. Louis, Missouri. $MgCl_2$ was purchased from Mallinckrodt, St. Louis, Missouri. ATP, creatine phosphokinase, creatine phosphate and unlabeled thymidine were purchased from Calbiochem, Los Angeles, California. Unlabeled folic acid (Folvite) and methotrexate (MTX) were obtained through the courtesy of Lederle, Pearl River, New York. 2,5-Diphenyloxazole, p-Bis[2-(5-phenyloxazolyl)]-benzene and thymidine-2-^{14}C (54 μCi/umole) were purchased from New England Nuclear, Boston, Massachusetts. ^{3}H-PGA (33 Ci/mmole and 29 Ci/mmole) was purchased from Amersham-Searle, Des Plaines, Illinois.

METHODS

I. Determination of mucosal to serosal folate transfer in the rat small intestine.

The two lots of ^{3}H-PGA labeled in the 3', 5' and 9 positions of the molecule (figure 6) were purified by column chromatography on A-25 DEAE-Sephadex (Pharmacia). The purified lots had specific activities of 5.6 Ci/mmole and 45.8 Ci/mmole respectively, and were greater than 99% radiochemically pure.

Mucosal to serosal transfer of ^{3}H-PGA and its derivatives were determined under short circuited conditions using the methods and apparatus described by Schultz and Zalusky[47]. Flat sheets of rat jejunum or ileum from male albino Sprague-Dawley

rats weighing 300-350 grams were mounted between lucite chambers. Equimolar amounts of unlabeled PGA at varying concentrations were placed on both the mucosal and serosal side of the chamber with approximately 3.0 μCi of ^{3}H-PGA added to the mucosal solution. After a 20 minute equilibration period, the rate of appearance of radiolabeled material in the serosal solution was determined by taking 1 ml aliquots from the serosal media at 20 minute intervals. The sample added to 10 ml of Bray's solution[48] was counted in a Packard Tri-Carb 3-Channel liquid scintillation spectrometer for 10 minutes. In these studies, results were expressed in pmoles of folate transferred to the serosal solution per cm^2 intestine per 100 minutes. All experiments were performed in the absence of light. Both sides of the tissue were bathed with identical solutions maintained at 37°C. and bubbled continously with O_2-CO_2 (95:5, v/v). In all experiments, the composition of the buffer bathing solution in millimoles per liter was sodium chloride, 115; sodium bicarbonate, 25; potassium phosphate, 5; magnesium chloride, 1.2; calcium chloride, 1.2; ascorbic acid, 17; pH 7.4.

II. Identification of folate compounds by chromatography.

At the end of 100 minutes, the serosal bathing solutions from two to three experiments were pooled and stored overnight in 3 M mercaptoethanol at 4°C. A similar procedure was followed for the mucosal bathing solutions. Folate compounds were chromatographically identified using the method described by Nixon and Bertino[49]. The pooled serosal or mucosal samples containing folate markers were diluted to a salt concentration of 0.1 molar

and applied to a 0.9 x 27 cm column of A-25 DEAE-Sephadex equilibrated with 0.1 M phosphate buffer, pH 6.0, containing 10 mM mercaptoethanol. Materials were eluted by phosphate buffer containing 10 mM mercaptoethanol, pH 6.0, the concentration of which was increased linearly from 0.1 M to 1.8 M. The final concentration of 1.8 M phosphate buffer was obtained after passage of 30 ml of eluting buffer. Non-radioactive markers of 5-MTHF, PGA and p-aminobenzoylglutamate (pABG) were added with the sample. Fractions of 2.5 ml were collected in the dark at 4°C. and monitored by ultraviolet absorbance spectra and by radioactivity. 0.5 ml aliquots per fraction were counted with the Packard Tri-Carb spectrometer in 15 ml of scintillation fluid containing 8.0 g of 2,5-Diphenyloxazole in 1 liter of absolute alcohol and 100 mg of p-Bis[2-(5-phenyloxazolyl)]-benzene in 2 liters of toluene. The compounds identified were quantitated in terms of pmoles of PGA or 5-MTHF transferred to the serosal solution per cm^2 intestine per 100 min by integrating the area under each peak in the chromatograph to determine the relative proportion of each compound transferred, and by combining this information with the total mucosal to serosal folate transfer obtained as described in section I. The average number of counts added to the column to identify the various folate compounds was between 25,000-30,000 cpm. At this level of activity, a 5-MTHF peak of at least 4% of the total folate activity can be identified. Therefore, in those experiments in which a 5-MTHF peak was not observed, we are assuming that less than 4% 5-MTHF

was present. Obviously, the correct amount of 5-MTHF is somewhere between 0 and 4%.

III. Preparation of rat small intestine for enzyme analysis and histology.

Male albino Sprague-Dawley rats weighing 200-250 g placed under ether anesthesia had their small bowel excised and placed in cold 0.5 x 10^{-4}M tris buffer with 10^{-4}M thymidine*, pH 7.5 (tris-thymidine buffer). The small intestine was divided into three equal parts of about 30 cm in length. The intestinal contents were removed from the proximal (jejunal) and distal (ileal) segments by flushing with cold tris-thymidine buffer and the middle segment was discarded. The jejunum was divided into three equal parts of about 10 cm in length and two of the three segments selected at random were lightly scraped along the mucosa according to the method of Dietchy and Siperstein[50] to separate the intestinal villi from the cryps-muscle layer. Jejunal villi scrapings were pooled and placed in 2-3 ml of tris-thymidine buffer. The third 10 cm segment underwent a more forceful scraping to separate total mucosa from the underlying

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*In preliminary experiments, it was found that the thymidine kinase assay could be performed on the intestinal homogenates only if the enzyme was stabilized by the presence of 10^{-4}M thymidine in tris buffer and if fresh supernatant fractions were utilized for the assay.

smooth muscle layer, after which the total mucosa was placed in 3-5 ml of tris-thymidine buffer. The same procedures were followed for the 30 cm ileal segment. The histology of these intestinal layers was determined on formalin fixed, hematoxylin and eosin and masson stained sections of each preparation (figure 9). The jejunal and ileal villi and total mucosa were each individually homogenized for 1-3 minutes with a glass motor-driven pestle in the initial volume of tris-thymidine buffer. The homogenates were then centrifuged for 15 minutes at 27,000 x g and the supernatant from each homogenate was divided into three aliquots. One aliquot was saved for the immediate determination of thymidine kinase activity and the other two were stored at -70°C. until used (usually within three days), for the determination of dihydrofolate reductase activity, sucrase activity and protein.

Thymidine kinase assay: The thymidine kinase activity of the fresh supernatant fraction of villi and total mucosa homogenates was determined using the assay of Chello and Jaffe[51] and the chromatographic separation of Furlong[52]. The thymidine kinase activity is expressed as pmoles of thymidine-5'-monophosphate formed per mg protein per 30 minutes.

Dihydrofolate reductase assay: The dihydrofolate reductase activity of the supernatant fractions of villi and total mucosa homogenates was determined at pH 7.5 at 37°C. using the method of Bertino, et. al.[53]. Activity is expressed as μmoles of dihydrofolate reduced per hour per mg protein.

Sucrase assay: The sucrase activity of the supernatant frac-

tions of villi and total mucosa homogenates was determined according to the method of Dahlqvist[54]. Activity is expressed as μg of glucose liberated per hour per mg protein.

Protein determination: Protein concentrations of the supernatant fractions of villi and total mucosa homogenates were determined by the biuret method with bovine serum albumin as the standard.

Statistics: Statistical significance was determined by the student *t* test[55].

RESULTS

The identification and quantitation of folate compounds absorbed by rat small intestine.

The DEAE-Sephadex chromatographic identification of folate compounds in the serosal bathing solutions of rat jejunum incubated with 7 nM PGA, 20 nM PGA, 20nM PGA with 10^{-6}M MTX, and with 2000 nM PGA and the ileum with 20 nM PGA are shown in figures 1-5. The folate transfer studies were combined with this chromatographic data to give the pmoles of 5-MTHF and PGA transferred per cm^2 intestine per 100 min as shown in table 1. The total folate transfer was found to be linearly related to mucosal PGA concentration (table 1 and figure 8).

It is seen that when the concentration of PGA in the mucosal bathing solution incubating the jejunum is increased from 7 nM to 2000 nM, the per cent of the total folate identified in the serosal bathing solution as 5-MTHF decreased from 46% to less than 4%. Further, the presence of 10^{-6}M MTX, a dihydrofolate reductase inhibitor, markedly decreased the appearance of 5-MTHF in the serosal solution when 20 nM PGA was present on the mucosal side of the jejunum. When 20 nM PGA was on the mucosal side of the ileum, less than 4% 5-MTHF was identified.

The pABG peak identified for the serosal media is the result of oxidative breakdown of PGA and reduced folates occurring under the incubating conditions employed in these experiments (figure 7). The possibility of selective pABG uptake by intestinal mucosa awaits further study.

Enzyme analysis of small intestine.

The thymidine kinase, DHF reductase and sucrase specific activities of the supernatant fractions of jejunal and ileal villi and total mucosa are shown in table 2. The purity of the villus preparation was confirmed by a five fold increase in sucrase specific activity and a twenty fold decrease in thymidine kinase specific activity when compared to total mucosa homogenates and by histology (figure 9). Although DHF reductase specific activity was similar in proximal and distal total mucosa homogenates ($P<0.2$), a three fold increase in DHF reductase specific activity was found in jejunal villi compared to ileal villi ($P<0.001$). On the other hand, the jejunal:ileal ratio of thymidine kinase and sucrase specific activities was identical for both total mucosa homogenates and villi.

DISCUSSION

These studies demonstrate that the reduction and methylation of PGA to 5-MTHF occur in the rat small intestine and that this conversion is significantly greater in the jejunum than in the ileum. It is noted that in no chromatogram (figures 1-5) were peaks of radioactivity identified which might prove to be intermediate compounds in the pathway of PGA's conversion to 5-MTHF. This suggests that the initial reduction of PGA to tetrahydrofolate (FH_4) by DHF reductase may be the rate limiting step in this pathway (figure 7). However the absence of these peaks may indicate that the 17 mM ascorbate in the serosal bathing solution was not in sufficiently high concentration to protect these intermediate compounds from oxidation, and/or that chromatographing these intermediates in amounts smaller than the assay system was capable of identifying may have resulted in their presence being masked.

That methotrexate, a competitive stoichiometric inhibitor[56] of DHF reductase, markedly diminished the percent PGA converted to 5-MTHF by the rat jejunum (figure 3) suggested to us that the differences in PGA reduction and methylation to 5-MTHF between jejunum and ileum might be explained by differences in DHF reductase activity in these two regions of small intestine. In experiments with supernatant fractions of total mucosa homogenates from jejunum and ileum, the differences in DHF reductase activity was not statistically significant ($P<0.2$). However, the crypt region within the mucosa of the small bowel, a region

of active DNA[57,58] and protein[57,59] synthesis, might be expected to contain this enzyme in sufficient activity to mask real differences in specific activity between jejunal and ileal villi, the tissue usually considered important in intestinal absorption. Villus homogenates prepared according to the method of Dietchy and Siperstein[50] did reveal a higher DHF reductase specific activity in jejunal compared to ileal villi ($P<0.001$). This is in contrast to findings of an identical jejunal:ileal ratio for thymidine kinase and sucrase specific activities in both total mucosa homogenates and villi (table 2).

The use of sucrase and thymidine kinase as enzyme markers to verify the purity of the villus preparation is based on the work of Van Genderen[60] and others[57,61,62], which dealt with sequential biochemical analyses from villus to crypt in the intestine of the rat and demonstrated villi:crypt ratios of 4.0 and 0.02 for sucrase[57] and thymidine kinase[62] respectively.

There is at least one study which by autoradiographic techniques[44] indirectly demonstrated DHF reductase activity in the villi of the proximal small bowel. Results of further experiments to determine if DHF reductase is localized to the microvilli of the brush border might suggest, as proposed by Crane[63], whether or not a "kinetic advantage" exists for the absorption of PGA similar to that which may exist for disaccharide absorption, on the basis of specific membrane-substrate-enzyme organization at the level of the microvillus. It would also be interesting to determine whether DHF reductase in the highly specialized villus has the same properties as DHF reductase in the relatively

undifferentiated crypt cell.

These results indicate that the transfer of PGA across the intestine is directly related to the mucosal PGA concentration (figure 8). However the percent PGA converted to 5-MTHF is not linearly related to PGA concentration: at 7 nM PGA, 46% is methylated, but at 2000 nM less than 4% can be identified as 5-MTHF in the serosal solution (table 1). This data can then be interpreted to indicate that PGA to 5-MTHF conversion requires a rate limiting step but that total PGA transfer does not take place in a saturable system (figure 8). One would like to speculate that the saturable process for PGA absorption observed by some investigators[16,20,21,23-25] is related to this rate limiting step of PGA conversion to 5-MTHF.

Therefore, what one finds in the serosal solution or prehepatic blood sample is dependent upon the concentration of PGA used in the mucosal incubating media, the surface area and time available for absorption, the quantity of folate in the sample to be analyzed and upon the sensitivity of the assay method employed. Previous experiments in the field which appeared to be utilizing quantities of PGA greater than 100 nM[20,35,45,46] would therefore not be expected to identify a significant percentage of 5-MTHF during absorption. Smith, Matty and Blair, in studies with everted intestinal sacs from rats[20] incubated with 10^{-7} to 10^{-6}M PGA, did report a "minor radioactive component" with their chromatographic assay system which cochromatographed with both 5-formyltetrahydropteroylglutamic acid and 5-MTHF markers. Also, the possibility that the measures taken in

these studies to protect reduced folates from light[35] and aerobic conditions[20,35,45,46] were not optimal may have contributed to the difficulty encountered by these workers in identifying absorbed 5-MTHF. Both Strum, et. al.[40] and Cohen[25], having used everted intestinal sacs from rats and hamsters respectively, found conversion of PGA to 5-MTHF during absorption. However, they did not study the characteristics of this conversion.

The mucosa to serosa transfer data for jejunum and ileum (table 1), derived from only two to three pooled experiments per study, does not have statistical validity for any conclusion to be drawn regarding a preferential site for folate absorption. Past clinical[11-15] and experimental[16-20] research favors the jejunum as the primary site of folic acid absorption although one study in the rat[21] finds no difference between jejunal and ileal PGA absorption.

The recent observation[64] that the small intestine of the rat is the only organ which lacks the 5-methyltetrahydrofolate-homocysteine methyltransferase enzyme for the conversion of homocysteine to methionine may indicate that in methionine metabolism there is a shunting of 5-MTHF, the predominant circulating and storage form of folate[65], via the blood stream to the liver, kidney, adrenals, testes, pancreas and brain, where in all these organs good specific activity for this enzyme has been found[64]. If other enzymes related to folate utilization are found to be lacking in the small intestine, it may suggest that the small intestine is an effective "scavenger" of dietary folate for preferential use elsewhere in the body.

Reports of a congenital isolated defect for folate absorption in man[66,67] offer a good opportunity for further elucidation of the specific uptake mechanisms for PGA by the mucosal cell.

LEGENDS

Figure 1. Chromatographic identification on DEAE-Sephadex of folate compounds found in the serosal bathing solution of rat jejunum mounted between lucite chambers and incubated with 7 nM PGA in the mucosal and serosal solutions with ^{3}H-PGA in the mucosal solution. Results of three pooled experiments. Radioactivity was identified in the 273, 280 and 289 mμ peaks which cochromatographs with unlabeled pABG, PGA and 5-MTHF. pABG, PGA and 5-MTHF were added to the column as markers.

Figure 2. Chromatographic identification on DEAE-Sephadex of folate compounds found in the serosal bathing solution of rat jejunum incubated with 20 nM PGA. Results of two pooled experiments. Further details are presented in figure 1 and in the text.

Figure 3. Chromatographic identification on DEAE-Sephadex of folate compounds found in the serosal bathing solution of rat jejunum incubated with 20 nM PGA and 10^{-6}M methotrexate. Results of two pooled experiments. Further details are presented in figure 1 and in the text.

Figure 4. Chromatographic identification on DEAE-Sephadex of folate compounds found in the serosal bathing solution of rat jejunum incubated with 2000 nM PGA. Results of two pooled experiments. Further details

are presented in figure 1 and in the text.

Figure 5. Chromatographic identification on DEAE-Sephadex of folate compounds found in the serosal bathing solution of rat ileum incubated with 20 nM PGA. Results of two pooled experiments. Further details are presented in figure 1 and in the text.

Figure 6. The structure of pteroic acid, folic acid and folic acid polyglutamates. Glutamic acid residues are gamma carboxy linked. Modified from Krumdieck and Baugh[68].

Figure 7. The pathway for the reduction and methylation of folic acid to 5-methyltetrahydrofolic acid.

Figure 8. Graphic representation of data from table 1 demonstrating the transfer of total folate (——) and 5-MTHF (----) from the mucosal to the serosal solution with increasing $[PGA]_{mucosal}$.

Figure 9. Histologic demonstration of villus scrapings prepared as described in the text. Only pure villi were identified in the villus scrapings.

figure 1.

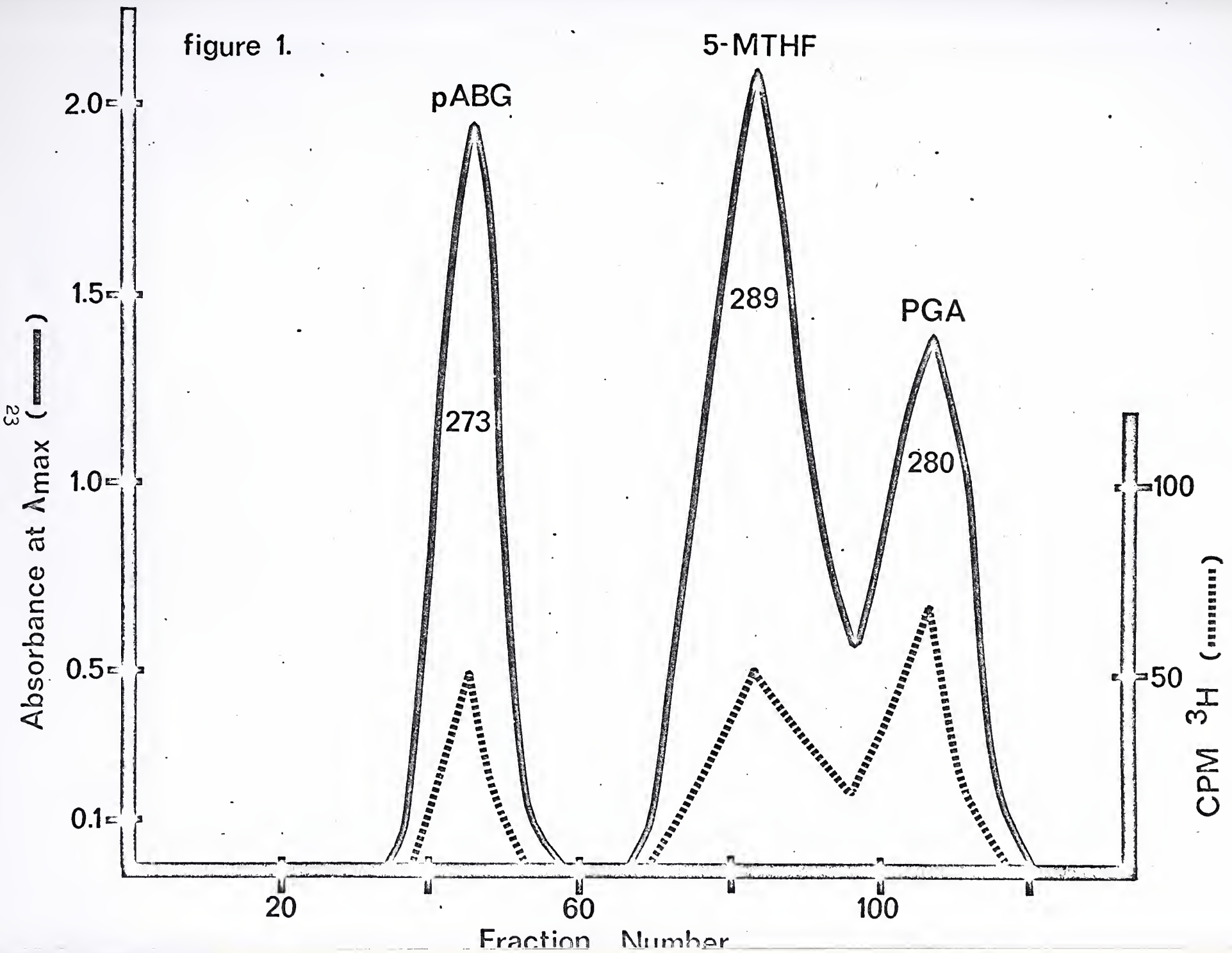
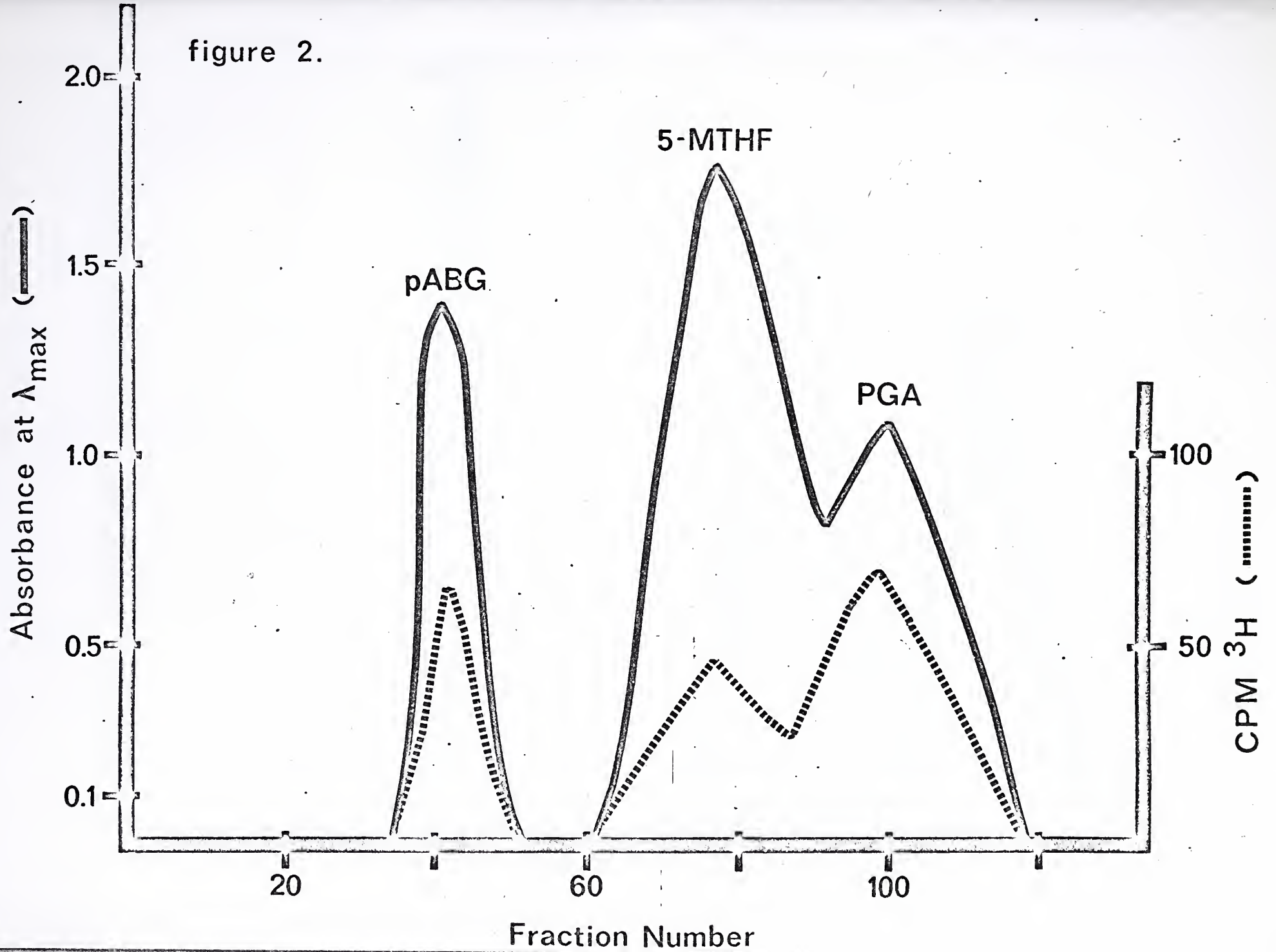

5-MTHF
pABG
PGA
289
273
280
2.0
1.5
1.0
0.5
0.1
Absorbance at λmax (——)
CPM ³H (·····)
100
50
20
60
100
Fraction Number

figure 2.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1.5
1.0
0.5
0.1
Absorbance at λ_{max} (——)
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PGA
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CPM ^3H (·····)
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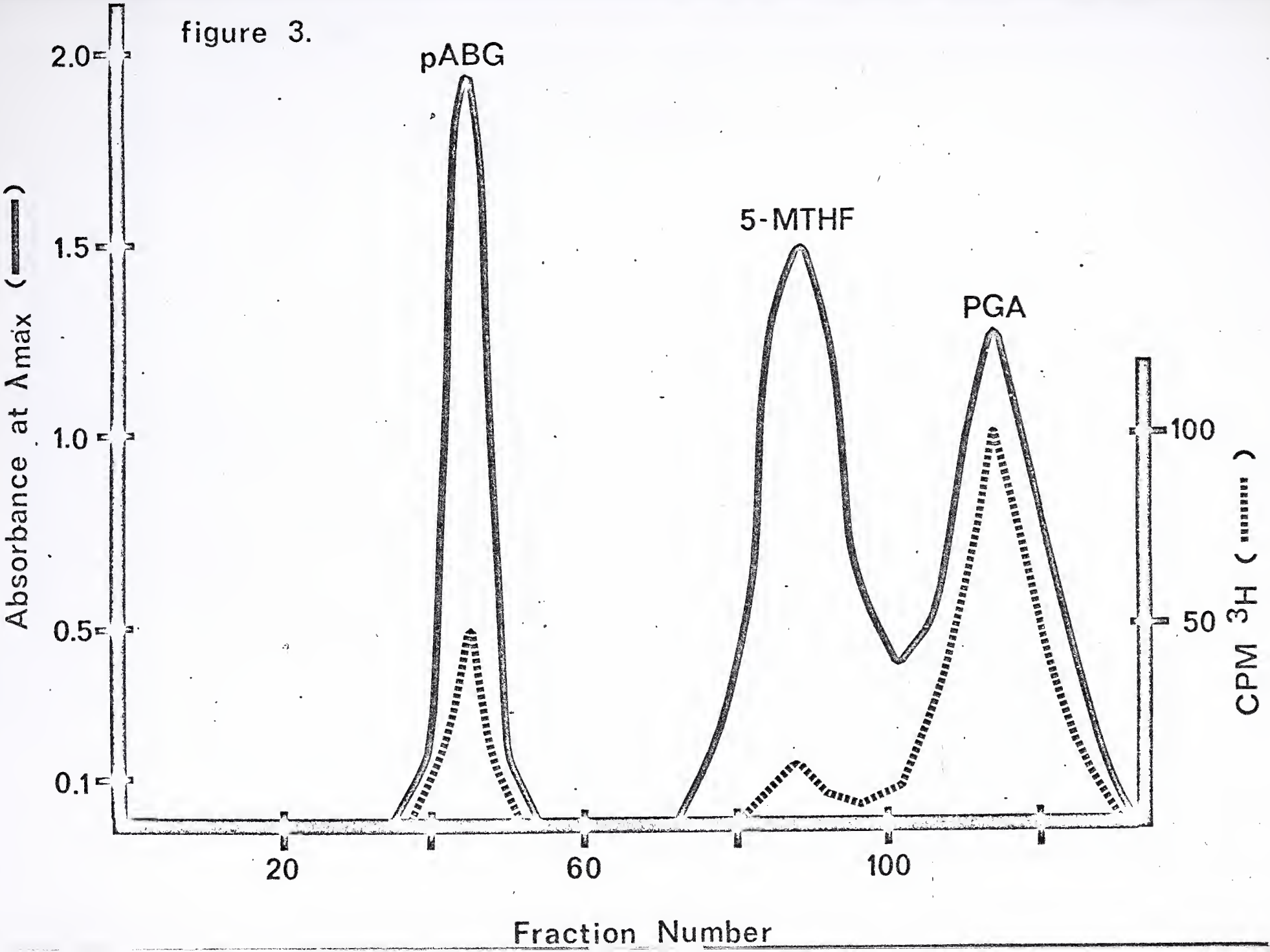
60
100
Fraction Number

figure 3.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PGA
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1.0
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0.1
Absorbance at λmax (——)
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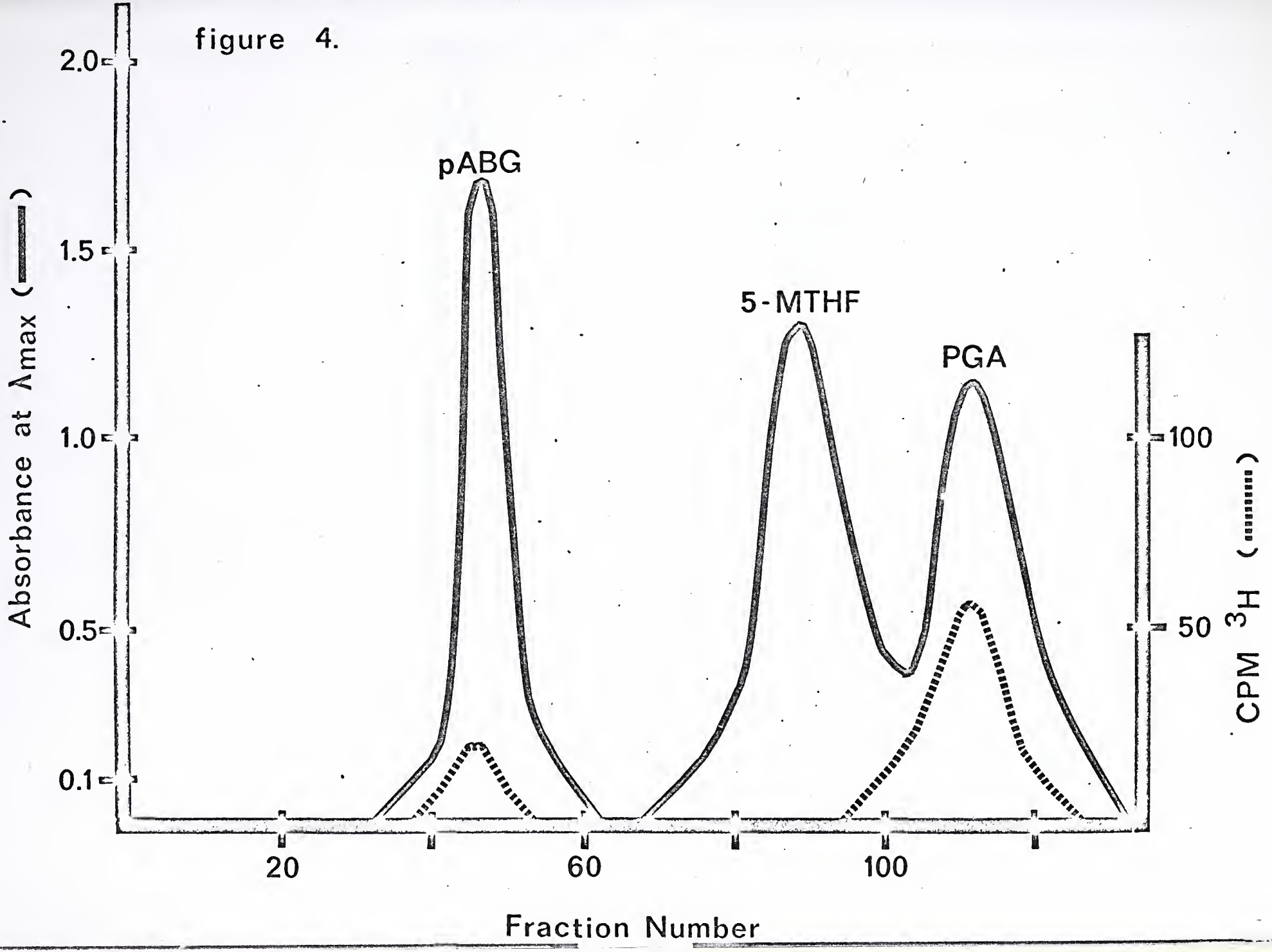
50
CPM ³H (·····)
20
60
100
Fraction Number

figure 4.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5-MTHF
PGA
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1.5
1.0
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0.1
Absorbance at λmax (——)
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50
CPM ^{3}H (.....)
20
60
100
Fraction Number

figure 5.

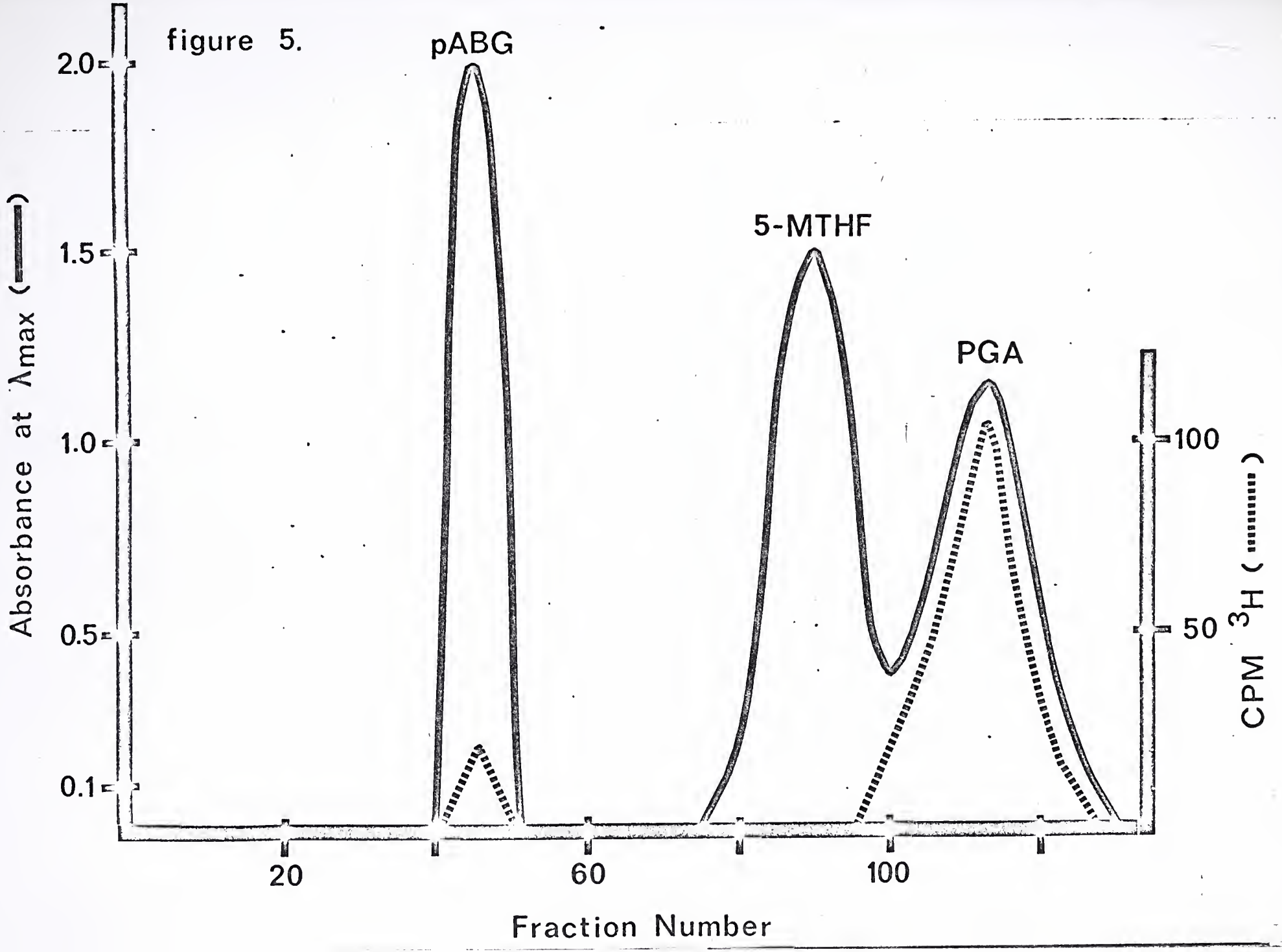
pABG
5-MTHF
PGA
2.0
1.5
1.0
0.5
0.1
Absorbance at λmax (——)
100
50
CPM ³H (·····)
20
60
100
Fraction Number

FIGURE 6.

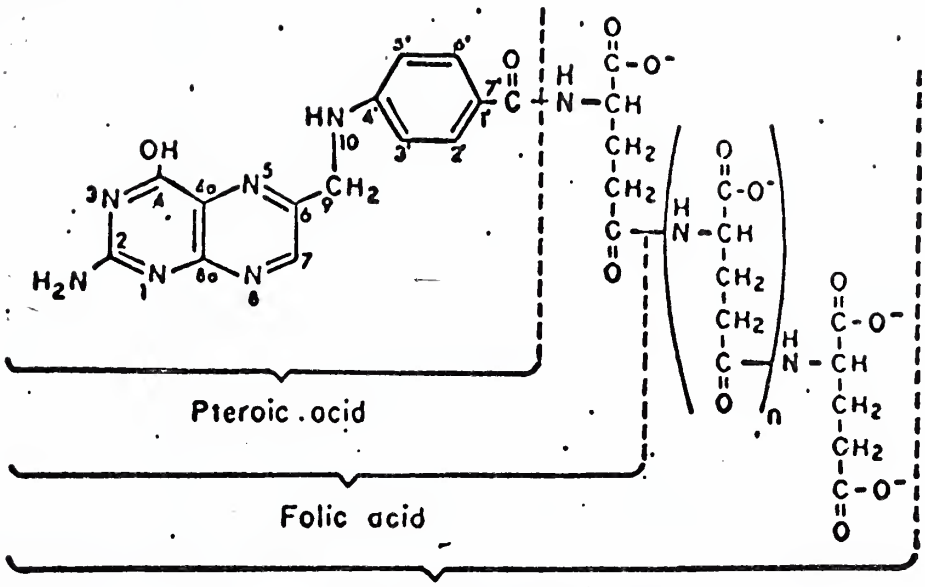

FIGURE 7.

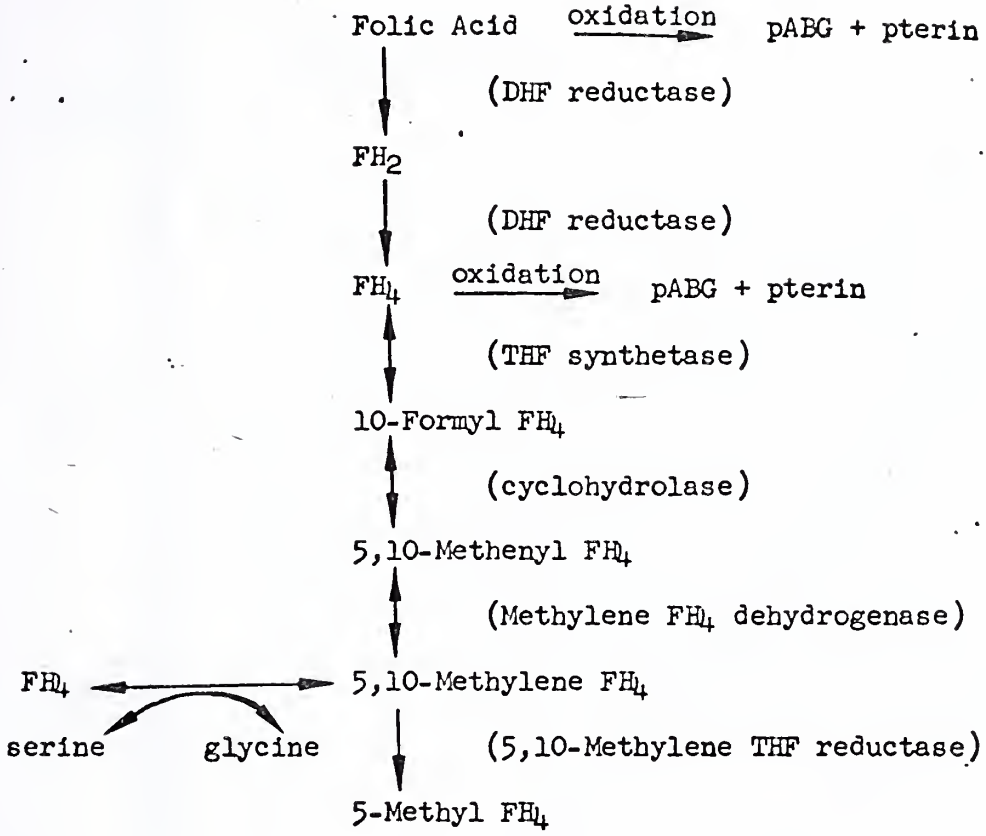
Folic Acid
oxidation
pABG + pterin
(DHF reductase)
FH_2
(DHF reductase)
FH_4
oxidation
pABG + pterin
(THF synthetase)
10-Formyl FH_4
(cyclohydrolase)
5,10-Methenyl FH_4
(Methylene FH_4 dehydrogenase)
FH_4
5,10-Methylene FH_4
serine
glycine
(5,10-Methylene THF reductase)
5-Methyl FH_4

figure 8.

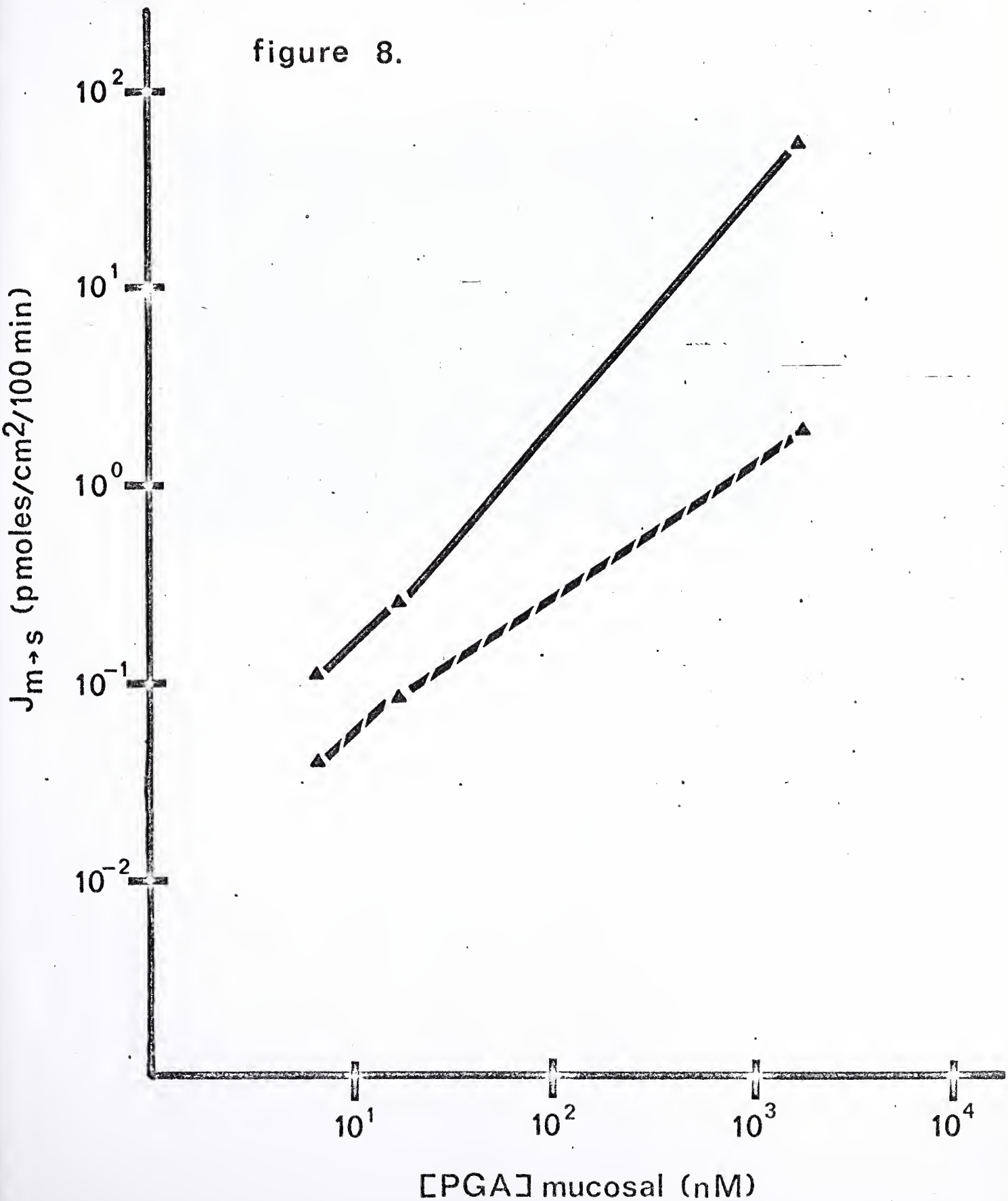
10^2
10^1
10^0
10^{-1}
10^{-2}
$J_{m \to s}$ (p moles/cm^2/100 min)
10^1
10^2
10^3
10^4
[PGA] mucosal (nM)

Figure 9.

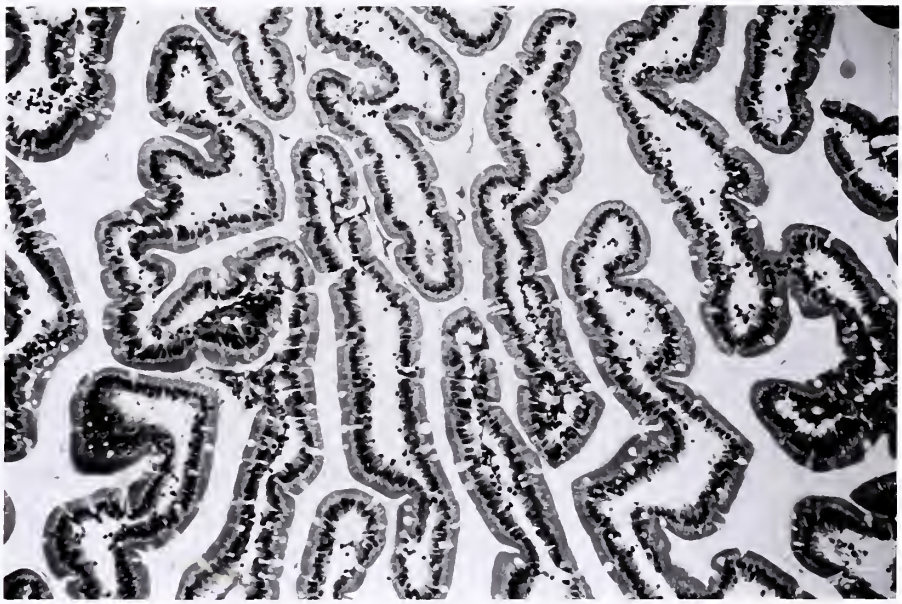

TABLE 1.

Jejunal Conversion of PGA to 5-MTHF

Study #	Tissue	nM PGA in mucosal solution	Total** Folate $J^{*}_{M \rightarrow S}$ (pm/cm^2/100 min)	% 5-MTHF of total $J^{Folate}_{M \rightarrow S}$	$J^{5\text{-MTHF}}_{M \rightarrow S}$ (pm/cm^2/100 min)	% PGA of total $J^{Folate}_{M \rightarrow S}$	$J^{PGA}_{M \rightarrow S}$ (pm/cm^2/100 mi
1	jejunum	7	0.103	46	0.047	54	0.056
2	jejunum	20	0.260	34	0.090	66	0.170
3	jejunum	20 plus 10^{-6}M MTX	0.424	6	0.026	94	0.398
4	jejunum	2000	47.738	<4	<1.910	>96	>45.828
5	ileum	20	0.557	<4	<0.022	>96	>0.535

** PGA and 5-MTHF

* Transfer from mucosal to serosal solution

TABLE 2.

DHF reductase, sucrase and thymidine kinase specific activities (S.A.) in the supernatant fractions of villi and total mucosa homogenates from rat jejunum and ileum (± S.E.)

Tissue (n=6)	DHF reductase S.A. (μmoles DHF reduced/ mg protein/hr at 37°C)	Sucrase S.A. (μg glucose liberated/mg protein/hr)	Thymidine kinase S.A. (pmoles TMP formed/mg protein/30 min)
jejunal villi	0.105* ± 0.012	68.20 ± 3.66	4.31 ± 0.91
jejunal total mucosa	0.235** ± 0.040	14.43 ± 2.77	115.90 ± 24.96
ileal villi	0.036 ± 0.003	67.82 ± 5.34	4.03 ± 0.89
ileal total mucosa	0.155 ± 0.030	14.87 ± 1.39	102.59 ± 11.74

** $p < 0.2$, compared to ileal total mucosa

* $p < 0.001$, compared to ileal villi

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